Using biochemical and electrophysiological methods we found that pentamidine exclusively inhibits hERG export from the endoplasmic reticulum to the cell surface in a heterologous expression system as well as in cardiomyocytes. hERG trafficking inhibition could be rescued in the presence of the pharmacological chaperone astemizole. We used rescue experiments in combination with an extensive mutational analysis to locate an interaction site for pentamidine at phenylalanine 656, a crucial residue in the canonical drug binding site of terminally folded hERG. Our data suggest that pentamidine binding to a folding intermediate of hERG arrests channel maturation in a conformational state that cannot be exported from the endoplasmic reticulum. We propose that pentamidine is the founding member of a novel pharmacological entity whose members act as small molecule 'anti-chaperones'.

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Association of Kv10.1 to Different Plasma Membrane Domains and its Interaction with other Membrane Associated Proteins in Endogenous Expression Systems

Aura M. Jiménez[1], Alicia Ortega[1], Luis A. Pardo[2], Walter Stühmer[2].
[1]UNAM, Mexico City, Mexico, [2]MPI-EM, Göttingen, Germany.

Kv10.1 is voltage gated potassium channel of the EAG family, which is mainly expressed in the Central Nervous System (CNS) under physiological conditions. In the past years, a causal association between Kv10.1 and tumor growth has also been proven, nevertheless the precise mechanism by which this occurs remains unknown. In this work we compare the direct association of membrane-associated proteins to Kv10.1 in different membrane domains of brain and cancer cells. Plasma membranes of brain tissue of adult mice (C57BL/6) and of the human prostate cancer cell line DU-145 were isolated. Detergent resistant membranes (DRM) were extracted through Triton X-100 solubilization and discontinuous gradient centrifugation. IP-assay for Kv10.1 was performed in the different membrane domains; protein expression was analyzed through the Colloidal Coomasie method. Kv10.1 associates partially to the DRM in brain tissue whereas in cancer cells no association can be observed. The protein pattern associated to Kv10.1 in the different systems also varies. We conclude that the different domain association of Kv10.1 is important for different protein interactions and could be involved in it's role as a tumor promoting protein.

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Slack Channels are Sensitive to External Sodium

Youshan Yang, Yangyang Yan, Fred J. Sigworth.
Yale University School of Medicine, New Haven, CT, USA.

The potassium channels formed by Slack (Slo2.2) subunits are activated by intracellular Na^+. In cell-free patch recordings from channels expressed in Xenopus oocytes we observe that the $K_{1/2}$ for activation by intracellular Na^+ decreases from about 80 to 35 mM when extracellular Na^+ is raised from 15 to 145 mM. This suggests several possibilities: that Na^+ sensing is influenced by extracellular sites on the channel; sensing involves the selectivity filter; or that Na^+ permeation leads to binding to the intracellular Na^+-sensor. Favoring the last possibility is the further observation of a ''latch state'' in which channels are irreversibly opened when intracellular K^+ is replaced entirely by Na^+.

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Mutant Analysis of the Determinants of Sperm Membrane Resting Potential

Julio César Chavez[1], Claudia L. Treviño[2], Alberto Darszon[2], José Luis de la Vega[2], Lawrence Salkoff[1], Celia M. Santi[1].
[1]Washington University School of Medicine, Saint Louis, MO, USA, [2]Instituto de Biotecnología, UNAM, Cuernavaca, Mexico.

The SLO3 pH-sensitive K+ channel is essential to fertilization. SLO3 is a unique member of the SLO family of high conductance K+ channels in that its expression is limited to spermatozoa. It was recently shown that SLO3 activation during capacitation leads to sperm membrane hyperpolarization. SLO3-/- male mice are infertile and they show deficits in capacitation-induced hyperpolarization, progressive motility and acrosome reaction.
Using SLO3 potassium channel knock-out mice we undertook a mutant analysis to determine the major determinants of membrane sperm resting potential and how membrane potential changes during capacitation. The analysis revealed that three types of ion channels were the major determinants of the resting potential. These constituted an amiloride-sensitive sodium channel and two types of potassium channels, the SLO3 high conductance K+ channel, and a channel carrying a potassium leak conductance. SLO3 channels were inactive prior to capacitation. Nevertheless, blocking the amiloride-sensitive sodium channels prior to capacitation resulted in a membrane selectively permeable to potassium ions due to the dominance of the potassium leak over all other conductances. After capacitation the activation of SLO3 K+ channels increases the membrane potassium conductance by approximately 150% which, in physiological media, results in a capacitation induced membrane hyperpolarization. No major contributions of Cl- or Ca2+ to sperm membrane resting potential were noted.
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Direct Inhibitory Effect of Fluoxetine on TREK-2 Channel

Dawon Kang, Eun-Jin Kim, Jaehee Han.
Gyeongsang National University, Jinju, Korea, Republic of.

Earlier studies have reported that channel activity of TREK-1, a member of two-pore domain K^+ (K_{2P}) channel family, is inhibited by antidepressants, such as fluoxetine, norfluoxetine, and paroxetine. However, TREK-2, which is present in the same family, has been less studied than TREK-1. Well-known properties of TREK-1 are also exhibited by TREK-2. This study was performed to identify whether and how TREK-2 channel is inhibited by fluoxetine treatment. In HEK-293A cells transfected with rat TREK-2 or mutants (deletion of the amino or carboxyl terminal domain: TREK-2 ΔN, TREK-2 ΔC, and chimeras) DNA. Fluoxetine significantly inhibited both TREK-1 and TREK-2 activities in a dose-dependent manner. Under whole-cell mode and excised mode, fluoxetine showed inhibitory effect on TREK-2 current, suggesting that fluoxetine may modulate TREK-2 either directly or indirectly with different mechanism. However, fluoxetine produced high effects on TREK-2 channel activity in inside-out patches containing TREK-2 compared to whole-cell and outside-out patches. To identify the regions responsible for fluoxetine effect, we studied the role of the cytoplasmic regions of TREK-2. TREK-2 ΔN had no effect on fluoxetine-induced TREK-2 inhibition. TREK-2 ΔC abolished the inhibition in response to fluoxetine. The regions that allow inhibition by fluoxetine were localized to a charged region near the proximal C-terminus (KKTKEE). The deletion of KKTKEE abolished sensitivity to fluoxetine. From these results, we suggest that fluoxetine may modulate TREK-2 directly through the C-terminus (in particular KKTKEE), which is found to be critical for their channel sensitivity to fluoxetine.

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Outwardly Rectifying Currents in Hepatocytes are Inhibited by 2-APB

Ricard Masia[1], Gary Yellen[2].
[1]Massachusetts General Hospital, Boston, MA, USA, [2]Harvard Medical School, Boston, MA, USA.

To investigate the electrophysiological properties of hepatocytes, we carried out whole-cell patch-clamp experiments using primary mammalian hepatocytes. We found that mouse, rat, and human hepatocytes exhibit characteristic currents that are activated upon dialysis of intracellular contents and comprise substantial current densities (mouse: 275 ± 44 pA/pF, n=10 cells; rat: 206 ± 35 pA/pF, n=15 cells; human: 258 ± 123 pA/pF, n=6 cells; V_m= +100 mV). The currents exhibit outward rectification (13.4 ratio of current at +100 mV over −100 mV), a reversal potential close to the hepatocyte resting membrane potential (−30 to −40 mV), and inhibition by 2-aminoethoxy diphenyl borate (2-APB) ($K_{1/2}$ = 18.4 ± 2.5 μM, n=5 mouse hepatocytes). The currents are also inhibited by clotrimazole (88.3 ± 5.1 % inhibition with 10 μM clotrimazole, n=4 mouse hepatocytes); are insensitive to 5 mM Ba^{2+}; and are largely insensitive to 5 mM TEA. The currents are also present in the human hepatocyte cell line HepG2, which is derived from the malignant liver tumor hepatocellular carcinoma (101 ± 31 pA/pF, n=9 cells; V_m= +100 mV). The currents in HepG2 cells additionally exhibit inward rectification at voltages more positive than +40 mV. This inward rectification is steeply voltage-dependent, suggesting block by intracellular cations that is not observed in primary hepatocytes. Additional experiments are pending to identify the molecular basis of these currents, as well as to elucidate their physiological role in the mammalian liver.

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STIM1 Mutants Modify CRAC by Altering Orai1 Protein Concentration

Tatiana Kilch[1], Dalia Al-Ansary[1], Grigori Rychkov[2], Christine Peinelt[1], Barbara A. Niemeyer[1].
[1]University of Saarland, Homburg, Germany, [2]Adelaide University, Adelaide, Australia.

The endoplasmic reticulum (ER) Ca(2+) sensor stromal interaction molecule 1 (STIM1) binds and activates plasma membrane localized Orai1 channels,